De ritis ratio and neutrophil-to-lymphocyte ratio in the diagnosis of COVID-19

Potential diagnostic laboratory parameters for COVID-19

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Abstract
Aim: The outbreak of the novel coronavirus disease (COVID-19) has been affecting the world day by day. The definitive diagnosis of COVID-19 is made using the real-time polymerase chain reaction (RT-PCR) method. Although the RT-PCR method is the gold standard for confirming infection, it requires a specialized laboratory, expensive equipment, and trained personnel. Thus, simple and alternative laboratory tests are needed to predict PCR positivity and disease.
Material and Methods: We analyzed laboratory parameters of 147 patients who were hospitalized with a pre-diagnosis of COVID-19 and sent RT-PCR samples. Of these, 76 tests were positive and 71 were negative. In addition to routine laboratory parameters, we also examined ratios derived from them, such as the De Ritis ratio (AST to ALT ratio) and Neutrophil- to- Lymphocyte Ratio (NLR). We investigated whether these parameters can predict the positivity or negativity of the PCR test.
Results: CRP, D-dimer, Ferritin, AST values, NLR, and De Ritis ratio were found to be significantly higher in the PCR- positive group. It was observed that the lymphocyte count was lower in the positive group.
Discussion: Our results suggest that routine laboratory tests that can be performed easily may also have the potential to predict COVID-19 positivity and negativity. These parameters can especially be used as an alternative in areas with a shortage of laboratories, equipment and personnel for PCR testing. Combining some hematological parameters and specific ratios derived from hematological parameters can help in identifying false positive/negative RT-PCR tests.

Keywords
De Ritis Ratio, Neutrophil-to-Lymphocyte Ratio, COVID-19

Introduction

Coronavirus Disease-2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), was first seen in December 2019 in Wuhan, China, and affects the world every day [1]. The definitive diagnosis of COVID-19 is made using the real-time polymerase chain reaction (RT-PCR) method, which is performed by amplifying viral RNA. Due to the rapid increase in the number of cases and their worldwide spread, it has been difficult to rapidly perform RT-PCR for every patient with symptoms or contact. To be able to apply the RT-PCR method, a specialized laboratory, expensive equipment, and trained personnel are required. It is a known fact that in some parts of the world, especially in developing countries, specialized laboratory facilities are limited. Even if the PCR test is performed in these specialized laboratories, the false negative rate is still around 20-30% [2-5]. Additionally, the PCR method is costly and takes 4 to 6 hours to obtain, process, and study a blood sample.

Major symptoms of SARS-CoV-2 include fever, dry cough, weakness, fatigue, and dyspnea [6,7]. Since the symptoms of COVID-19 are similar to those of other respiratory viral diseases, there has been an increased need for laboratory parameters that can predict a positive PCR result. Although there are many publications on the effects of laboratory parameters, especially acute phase reactants, on clinical outcomes in COVID-19 patients, studies on their value in predicting PCR positivity are limited [6].

In our study, we aimed to analyze laboratory parameters of 147 patients who were hospitalized with a pre-diagnosis of COVID-19 and sent RT-PCR samples. Of these, 76 tests were positive and 71 were negative. In addition to routine laboratory parameters, we also examined ratios derived from them, such as the De Ritis ratio (AST to ALT ratio) and Neutrophil- to-Lymphocyte Ratio (NLR). We investigated whether these parameters can predict a positive or negative PCR test result.

Material and Methods

Patient selection:

Ethical approval for this stuy was obtained from the local ethics committee (No: 2020/770). Patients, who were admitted to our clinic with a pre-diagnosis of COVID-19 between March 1, 2020, and August 31, 2020, who had complete clinical data and over 18 years of age, who had at least one of the symptoms such as fatigue, fever, cough, and dyspnea were included in the study. An RT-PCR sample for COVID-19 was taken from each patient. Patients with any infection other than SARS-CoV-2 that could affect neutrophil and lymphocyte counts, and

patients with any disease that might affect AST, ALT levels, and thus AST/ALT ratio, were not included in the study. As a result of screening based on the above criteria, 31 out of 178 patients were excluded from the study. Of those, 28 had deficiencies in laboratory parameters and medical history, and one of them was under 18 years of age. Two patients were transferred to the intensive care unit due to a deterioration of their general condition on the day of hospitalization. The study included 147 patients (Figure 1).

Data collection and assessments:

The data were scanned retrospectively. Age, gender, symptoms, and findings of the patients at the time of diagnosis were recorded. The levels of neutrophils, lymphocytes, AST, ALT, C-reactive protein (CRP), ferritin, D-Dimer, lactate dehydrogenase (LDH), hemoglobin, platelets, monocytes were examined simultaneously with the RT-PCR sample taken for the diagnosis of COVID-19. The NLR and De Ritis ratios were calculated. Laboratory data of 76 patients with positive RT-PCR results and 71 patients with negative results were compared in terms of assessing the positivity or negativity of the PCR test.

Statistical Analysis:

Discrete variables, numbers and ratios were used for descriptive statistics. For continuous variables with normal distribution, standard deviation was preferred. The Chi-square test was used to compare groups involving discrete variables. $P < 0.05$ was accepted as statistically significant. All statistical analyzes were performed using SPSS (Statistical Packages for the Social Sciences) software version 22.0 (Chicago, IL, USA).

Results

The study included 147 cases, 76 of which tested positive for COVID-19 whereas 71 were negative. It was observed that the mean age of the positive group (59.96 years) was higher than that of the negative group. When patients' complaints upon presentation to the hospital were questioned, the most common symptoms and signs were cough and fatigue in the positive group, and fatigue, cough, and fever in the negative group, respectively. Gastrointestinal symptoms were least common in both groups (Table 1).

The AST value was found to be significantly higher in the PCR-positive group (p=0.013). On the other hand, ALT values did not differ significantly between the two groups. Whereas, the De Ritis ratio was higher in the PCR-positive group (p=0.002).

When the neutrophil and lymphocyte counts of the cases were examined, it was observed that the lymphocyte count was lower in the positive group, and there was a significant difference between the groups (p=0.002). Lymphopenia was seen in 73.6% (n=56) of positive cases. This rate was 47.8% (n=34) in negative cases. There was no significant difference between neutrophil values. The NLR value was higher in the positive group (p=0.035). CRP (p<0.001) and D-dimer (p <0.001) values were also statistically significantly higher in the positive group. The mean of ferritin values of COVID-19 positive cases was found to be close to 2 times the average of the negative group, and a significant difference was observed (p=0.005). There was no significant difference between the groups for LDH, platelet, monocyte, and hemoglobin values, respectively (p=0.662, p=0.084, p=0.318, p=0.955).
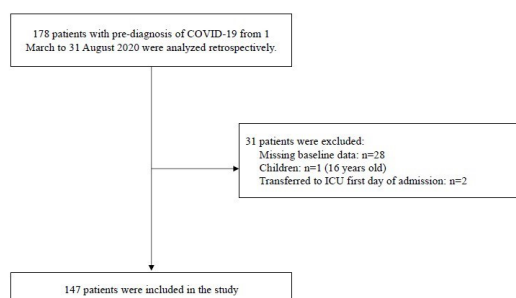

Figure 1. Study population

Table 1. Demographic characteristics of the study patients

	COVID-19 positive (n=76)	COVID-19 negative (n=71)
Gender		
Male (n,%)	41 (53.9)	41 (73.1)
Female (n,%)	35 (46.1)	30 (26.9)
Age (years±STD)	59,96±17,26	54,17±19,36
Sign and symptoms		
Cough (n,%)	58 (76.3)	31 (43.6)
Fatigue (n,%)	53 (69.7)	45 (63.3)
Fever (n,%)	43 (56.5)	31 (43.6)
Dyspnea (n,%)	22 (28.9)	8 (11.2)
Gastrointestinal symptoms (n,%)	13 (17.1)	7 (9.8)

Table 2. Average laboratory findings and corresponding standard deviation

Parameters	COVID-19 positive (n=76)	COVID-19 negative (n=71)	p-Value
AST (U/L)	64.04±73.8	54.17±19.36	0.013
ALT (U/L)	34.34±28.51	32.63±44.49	0.780
De Ritis Ratio	1.79±0.91	1.36±0.72	0.002
Neutrophil count (x109 cells/L)	5.92±4.33	5.65±3.58	0.684
Lymphocyte count (x109 cells/L)	1.26±0.66	1.63±0.77	0.002
NLR	6.48±7.70	4.38±3.61	0.035
CRP (mg/L)	64.34±54.00	32.96±37.30	<0.001
Ferritin (mcg/L)	401.34±429.74	240.04±224.24	0.005
D-Dimer (mcg/L)	679.25±710.89	357.49±261.34	<0.001
LDH (U/L)	302.79±197.94	282.70±343.41	0.662
Hemoglobin (g/L)	12.09±2.47	12.06±2.46	0.955
Platelets (x109 cells/L)	261.71±120.78	232.63±78.17	0.084
Monocytes (x109 cells/L)	0.63±0.39	0.69±0.34	0.318

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, NLR: Neutrophil-to-Lymphocyte Ratio, De Ritis Ratio: AST-to-ALT Ratio

Discussion

Of the 147 patients included in our study, more than half of all cases were males. More than half of the cases in the PCR (+) group were also males. In the descriptive studies by Chen et al. with 99 patients and Wang et al. with 138 patients, the number of male patients was found to be higher than that of females [1,8]. In our study, the median age of all patients was 57.1 years old, which is close to the data reported by Chen et al (55.5 years) and Zhang et al (57 years) [1,6]. Fever, cough, and fatigue were the dominant symptoms of the RT-PCR- positive group in our study. In a study by Liu et al. with 245 patients, the most common symptoms and signs were fever, cough, and fatigue [9].

In our study, a significant and strong relationship was found between AST, one of the transaminases, and PCR positivity (p=0.013). In a study by Ferrari et al., it was found that there is a strong relationship between the elevation of serum transaminases (AST and ALT) and the presence of COVID-19 [10]. However, in our study, no significant relationship was observed between the ALT elevation and PCR positivity (p=0.780). AST values were found to be significantly higher in the COVID-19 group in the study by Zhang et al., in which they examined 115 patients and compared COVID-19 with community-acquired pneumonia [11]. In a review by Feng et al., it was stated that the

AST value was more affected and increased by liver damage and dysfunction due to COVID-19. Although the pathophysiological mechanisms of this increase have not been fully clarified, it has been suggested that the possible mechanisms may be systemic inflammatory response, multiple organ dysfunction, and hypoxia caused by respiratory distress syndrome, which can be seen in the course of the disease [12]. The De Ritis ratio, known as an AST to ALT ratio, has been studied in many clinical conditions since it was defined by Fernando De Ritis in 1957 and has been shown in some studies to be an indicator of the inflammatory process and a parameter that affects survival in malignant processes [13,14]. In our study, the De Ritis ratio was found to be significantly higher in the PCR positive group (p=0.002). This result suggested that the De Ritis ratio may have the potential to predict PCR positivity of cases at the time of their first admission.

In the studies by Zhang et al. with 140 patients and Shi et al. with 90 patients, severe and non-severe patient groups were compared and it was found that the severity of the disease was associated with the low lymphocyte count (p=0.048, p<0.001) [6,15]. Ferrari et al. found that lymphocyte counts were lower in patients who presented with COVID-19 symptoms and tested positive with reverse RT-PCR than in the negative group (p=0.01) [10]. In our study, the results were consistent with the literature. Lymphocyte counts in the PCR-positive group were numerically lower than in the negative group and a statistically significant difference was found (p=0.002). However, the neutrophil counts of the patients were similar in both groups (p=0.684). Ferrari et al. found that the neutrophil count was lower in the PCR-positive group [10]. In the study by Cheng et al., it was observed that the average number of neutrophils in the COVID-19 and non-COVID-19 groups were similar to each other as in our study (p = 0.14) [16].

In the study by Yormaz et al., it was observed that more than half of PCR- positive patients (53%) had a high NLR [17]. In a study conducted by Shi et al., NLR was examined between the severe and non-severe groups and was found to be higher in the severe group (p<0.001) [15.] In our study, the NLR rates in the PCR-positive group were found to be significantly higher than in the negative group (p=0.035). There are publications in the literature showing that NLR and neutrophil counts were detected to be higher and lymphocyte count to be lower in severe disease, but there are no publications directly investigating that these parameters can be used as markers to predict PCR-positive cases.

We observed no association between hemoglobin, platelet, monocyte levels, and PCR positivity (Table 2). Ferrari et al. found that there was a strong relationship between COVID-19 and low monocyte levels (p=0.001), but not with platelets [10]. Cheng et al. observed that platelet counts were lower in the COVID-19 group compared to the non-COVID-19 group (p = 0.00) [16].

When we examined the levels of CRP, D-dimer, and ferritin in our study, all of these parameters were found to be statistically significantly higher in the PCR-positive group compared to the negative group (p<0.001, p<0.001, and p=0.005). In the study by Ferrari et al., a significant difference was found between the groups with and without COVID-19 when CRP values were

examined (p=0.034) [10]. In the study conducted by Zhang et al., it was found that the high CRP and D-dimer levels were associated with the severity of the disease (p<0.001 and p<0.001) [6]. Also, in another study conducted by Shi et al. comparing laboratory parameters between severe and non-severe groups, a relationship was found between high CRP and D-dimer levels and severe disease (p<0.001 and p<0.001) [15]. Ferritin, which is a positive acute-phase reactant, increases in COVID-19, as in other inflammatory diseases. Studies have stated that ferritin may reach very high levels during COVID-19. In the study by Liu et al., the protein structure of SARS-CoV-2 was examined and it was observed that ORF1ab, ORF10, and ORF3a proteins attacked the heme part of the beta-1 chain of hemoglobin. As a result, it was observed that iron was dissociated from porphyrin. In light of these data, it was thought that ferritin values increased more during COVID-19 than during a normal inflammation [18]. There is no publication in the literature on the predictive value of ferritin levels for PCR positivity. In a retrospective cohort study conducted by Guan et al. with 1270 COVID-19 patients, the relationship between ferritin and mortality was examined, and ferritin was accepted as a significant predictor [19]. In another study examining 100 COVID-19 patients followed in the intensive care unit, ferritin levels were compared between the survivor and non-survivor groups, and the median ferritin concentration was about three times higher in the non-survivor group than the survival group (1722.25μg / L vs. 501.90μg / L, p <0.01) [20].

The most striking limitation of our study is the number of patients. Considering the increasing number of positive COVID-19 cases all over the world, the number of our patients is low. More cases and multi-center studies are needed to support our findings.

Conclusion

In our study, we examined the predictability of laboratory parameters and some specific ratios for the RT-PCR result. We suggest that high AST, CRP, ferritin, D-dimer values, NLR, De Ritis ratio, and low lymphocyte counts at the time of admission could predict PCR positivity. Although the RT-PCR test is the gold standard, routine laboratory tests that can be easily performed may also have the potential to predict COVID-19 positivity and negativity. These parameters can especially be used as an alternative in areas with a shortage of laboratories, equipment, and personnel for PCR testing.

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